IN THE CLAIMS:

Claims 1-242 are pending. With the entry of this amendment, claims 22, 23, 25-53, 58-64, 73-74, 80-87, 91-106, 109, 114, 116-120,123-175, 177-178, 180-185, 187-201, 203-206, 209- 211, 216-224, 226-237, 240-241 will be canceled. Claims 88-90, 107, 176, 179, 186, 202, 208, 213, and 215 will be amended. Claims 1-21, 24, 54-57, 65-72, 75-79, 88-90, 107-108, 110-113, 115, 121-122, 176, 179, 186, 202, 207-208, 212-215, 225, and 238-239 will remain pending. Please amend claims 1-242 to read as follows. This listing of claims will replace all prior versions, and listings, of claims in the application.

1. (original): A modified stem cell comprising a plurality of chromosomes and at least a first heterologous nucleic acid molecule,
   (a) wherein the modified stem cell can differentiate into a plurality of cell types; the first heterologous nucleic acid molecule is integrated into a chromosome of the modified stem cell at a first locus whereby, upon differentiation of the modified stem cell, the first heterologous nucleic acid is expressed into each of the cell types;
   (b) wherein the first heterologous nucleic acid molecule encodes a first polypeptide selected from secreted proteins, extracellular domains of transmembrane proteins, and active fragments thereof; and
   (c) wherein the first polypeptide is other than beta-galactosidase and a recombinase.
2. (original): A modified stem cell comprising a plurality of chromosomes and at least a first heterologous nucleic acid molecule,
   (a) wherein the modified stem cell can differentiate into a plurality of cell types; the first heterologous nucleic acid molecule is integrated into a chromosome of the modified stem cell at a first locus whereby, upon differentiation of the

modified stem cell, the first heterologous nucleic acid is expressed in the plurality of differentiated cell types;

(b) wherein the first heterologous nucleic acid molecule encodes a first polypeptide selected from single transmembrane proteins, multi-transmembrane proteins, kinases, proteases, phosphatases, phosphodiesterases, kinesins, histone deacetylases, hormone receptors, ubiquitin E3 ligases, and active fragments thereof; and

(c) wherein the first polypeptide is other than beta-galactosidase and a recombinase.

3. (original): A modified stem cell comprising a plurality of chromosomes and at least a first heterologous nucleic acid molecule,

(a) wherein the modified stem cell can differentiate into a plurality of cell types; the first heterologous nucleic acid molecule is integrated into a chromosome of the modified stem cell at a first locus whereby, upon differentiation of the modified stem cell, the first heterologous nucleic acid is expressed in the plurality of differentiated cell types;

(b) wherein the first heterologous nucleic acid molecule encodes a first polypeptide that is an episomal plasmid maintenance molecule or an active fragment thereof, and

(c) wherein the first polypeptide is other than beta-galactosidase and a recombinase.

4. (original): The modified stem cell of any of claims 1, 2, or 3, wherein the stem cell is selected from an embryonic stem cell or an adult stem cell.

5. (original): The modified stem cell of any of claims 1, 2, or 3, wherein the stem cell is an animal stem cell.

6. (original): The modified stem cell of claim 5, wherein the animal stem cell is a mouse stem cell.

7. (original): The modified stem cell of claim 5, wherein the animal stem cell is a human stem cell.

8. (original): The modified stem cell of claim 6, wherein the animal stem cell is a mouse embryonic stem cell.

9. (original): The modified stem cell of any of claims 1, 2, or 3, wherein the first locus is selected from ROSA26, ROSA5, ROSA11, and G3BP(BT5).

10. (original): The modified stem cell of claim 9, wherein the first locus is ROSA26.

11. (original): The modified stem cell of claim 1, wherein the first polypeptide is selected from one or more growth factors, differentiation factors, anti-differentiation factors, colony stimulating factors, cytokines, lymphokines, anti-inflammatory molecules, apoptotic and other anti-cancer molecules, anti-apoptotic molecules, proteins involved in signaling pathways, antibodies, and active fragments thereof.

12. (original): The modified stem cell of claim 11, wherein the first polypeptide is a protein involved in a signaling pathway, and the signaling pathway is a Wnt pathway.

13. (original): The modified stem cell of either of claims 1 or 2, wherein the first polypeptide is selected from a ligand and a receptor.

14. (original): The modified stem cell of claim 13, wherein the ligand is a Wnt ligand and the receptor is a Wnt receptor.

15. (original): The modified stem cell of either of claims 1 or 2, wherein the first heterologous nucleic acid molecule encodes a human protein or an active fragment thereof.

16. (original): The modified stem cell of claim 3, wherein the stem cell further comprises an episomal vector.

17. (original): The modified stem cell of claim 16, wherein the episomal maintenance molecule is a polyoma large T antigen when the episomal vector comprises a polyoma origin of replication.

18. (original): The modified stem cell of claim 16, wherein the episomal vector comprises a second heterologous nucleic acid molecule.

19. (original): The modified stem cell of claim 18, wherein the second heterologous nucleic acid molecule encodes a second polypeptide selected from secreted proteins, extracellular domains of transmembrane proteins, and active fragments thereof.

20. (original): The modified stem cell of claim 18, wherein the second heterologous nucleic acid molecule encodes a second polypeptide selected from single transmembrane proteins, multi-transmembrane proteins, kinases, proteases, phosphatases, phosphodiesterases, kinesins, histone deacetylases, hormone receptors, and ubiquitin E3 ligases.
21. (original): The modified stem cell of claim 18, wherein the second nucleic acid molecule is an RNAi molecule.
22. (canceled)
23. (canceled)
24. (original): The modified stem cell of claim 18, wherein the episomal vector further comprises a promoter that regulates the expression of the second heterologous nucleic acid molecule.
25. (canceled)
26. (canceled)
27. (canceled)
28. (canceled)
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42. (canceled)

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46. (canceled)

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48. (canceled)

49. (canceled)

50. (canceled)

51. (canceled)

52. (canceled)

53. (canceled)

54. (original): A non-human chimeric animal developed from a modified blastocyst comprising a blastocyst from a first animal that comprises a modified stem cell from a second animal or a progeny thereof,

wherein the modified stem cell comprises a stem cell that comprises a plurality of chromosomes and at least a first heterologous nucleic acid molecule,

wherein the modified stem cell can differentiate into a plurality of cell types; the first heterologous nucleic acid molecule is integrated into a chromosome of the modified stem cell at a first locus whereby, upon differentiation of the modified stem cell, the first heterologous nucleic acid is expressed in the plurality of differentiated cell types,

wherein the first heterologous nucleic acid molecule encodes a first polypeptide selected from secreted proteins, extracellular domains of transmembrane proteins, and active fragments thereof, and

wherein the first polypeptide is other than beta-galactosidase and a recombinase.

55. (original): A non-human chimeric animal developed from a modified blastocyst comprising a blastocyst from a first animal that comprises a modified stem cell from a second animal or a progeny thereof,

wherein the modified stem cell comprises a plurality of chromosomes and at least a first heterologous nucleic acid molecule,

wherein the modified stem cell can differentiate into a plurality of cell types; the first heterologous nucleic acid molecule is integrated into a chromosome of the modified term cell at a first locus whereby, upon differentiation of the modified stem cell, the first heterologous nucleic acid is expressed in the plurality of differentiated cell types,

wherein the first heterologous nucleic acid molecule encodes a first polypeptide selected from single transmembrane proteins, multi-transmembrane proteins, kinases, proteases, phosphatases, phosphodiesterases, kinesins, histone deacetylases, hormone receptors, ubiquitin E3 ligases, and active fragments thereof, and

wherein the first polypeptide is other than beta-galactosidase and a recombinase.

56. (original): A non-human chimeric animal developed from a modified blastocyst comprising a blastocyst from a first animal that comprises a modified stem cell from a second animal or a progeny thereof,

wherein the modified stem cell comprises a stem cell that comprises a plurality of chromosomes and at least a first heterologous nucleic acid molecule,

wherein the modified stem cell can differentiate into a plurality of cell types; the first heterologous nucleic acid molecule is integrated into a chromosome of the modified stem cell at a first locus whereby, upon differentiation of the modified stem cell, the first heterologous nucleic acid is expressed in the plurality of differentiated cell types,

wherein the first heterologous nucleic acid molecule encodes a first polypeptide that is an episomal plasmid maintenance molecule or an active fragment thereof, and

wherein the first polypeptide is other than beta-galactosidase and a recombinase.

57. (original): The non-human chimeric animal of claim 56, wherein the modified stem cell further comprises an episomal vector.

58. (canceled)

59. (canceled)

60. (canceled)

61. (canceled)

62. (canceled)

63. (canceled)

64. (canceled)

65. (original): A tissue obtained from the non-human chimeric animal of any one of claims 54, 55, or 56.

66. (original): The tissue of claim 65, selected from heart, lung, kidney, liver, brain, bone marrow, blood, bone, cartilage, prostate, ovary, skin, spinal cord, thymus, spleen, muscle, stomach, intestine, and pancreas.

67. (original): A cell derived from the tissue of claim 65.

68. (original): A cell obtained from the non-human chimeric animal of any one of claims 54, 55, and 56, wherein the cell is selected from heart cells, lung cells, kidney cells, liver cells, brain cells, bone marrow cells, blood cells, bone cells, cartilage cells, prostate cells, ovary cells, skin cells, spinal cord cells, thymus cells, spleen cells, muscle cells, stomach cells, intestinal cells, and pancreatic cells.

69. (original): The non-human chimeric animal of any of claims 54, 55, and 56, wherein the blastocyst is a blastocyst of an animal model of a human disease, disorder, syndrome, or condition.

70. (original): The non-human chimeric animal of claim 69, wherein the disease, disorder, syndrome, or condition is selected from an immune system disease, disorder, syndrome, or condition, a metabolic system disease, disorder, syndrome, or condition, a central nervous system disease, disorder, syndrome, or condition, and cancer.

71. (original): The non-human chimeric animal of claim 69, wherein the animal model of a human disease, disorder, syndrome, or condition is selected from a SCID mouse, a NOD mouse, a knockout mouse, a Rb -/- mouse, a p53 -/- mouse, a mouse that over-expresses human Aβ, and a mouse that over-expresses TGFβ.

72. (original): A differentiated cell, wherein the cell differentiates from the modified stem cell of any one of claims 1, 2, or 3.

73. (canceled)

74. (canceled)

75. (original): A non-human transgenic animal that is produced from a cross between two chimeric animals of any one of claims 54, 55, or 56, or a progeny thereof wherein the transgenic animal is homozygous for the first heterologous nucleic acid molecule.

76. (original): A composition comprising a plurality of the modified stem cells of any of claims 1, 2, or 3.

77. (original): A method of making a modified stem cell, comprising the steps of:

(a) obtaining a stem cell;

(b) obtaining a first heterologous nucleic acid molecule;

(c) targeting the first heterologous nucleic acid molecule for integration into a chromosome of the stem cell; and

(d) selecting a modified stem cell that comprises the first heterologous nucleic acid molecule,

wherein the first heterologous nucleic acid molecule encodes a first polypeptide selected from secreted proteins, extracellular domains of transmembrane proteins, and active fragments thereof, and

wherein the first polypeptide is other than beta-galactosidase and a recombinase.

78. (original): A method of making a modified stem cell, comprising the steps of:

(a) obtaining a stem cell;

(b) obtaining a first heterologous nucleic acid molecule;

(c) targeting the first heterologous nucleic acid molecule for integration into a chromosome of the stem cell; and

(d) selecting a modified stem cell that comprises the first heterologous nucleic acid molecule,

wherein the first heterologous nucleic acid molecule encodes a first polypeptide that is selected from single transmembrane proteins, multi-transmembrane proteins, kinases, proteases, phosphatases, phosphodiesterases, kinesins, histone deacetylases, hormone receptors, ubiquitin E3 ligases, and active fragments thereof, and

wherein the first polypeptide is other than beta-galactosidase and a recombinase.

79. (original): A method of making a modified stem cell, comprising the steps of:

(a) obtaining a stem cell;

(b) obtaining a first heterologous nucleic acid molecule;

(c) targeting the first heterologous nucleic acid molecule for integration into a chromosome of the stem cell; and

(d) selecting a modified stem cell that comprises the first heterologous nucleic acid molecule,

wherein the first heterologous nucleic acid molecule encodes a first polypeptide that is an episomal maintenance molecule or an active fragment thereof, and

wherein the first polypeptide is other than beta-galactosidase and a recombinase.

80. (canceled)

81. (canceled)

82. (canceled)

83. (canceled)

84. (canceled)

85. (canceled)

86. (canceled)

87. (canceled)

88. (currently amended): A method of making a ~~non-human~~ chimeric animal comprising the steps of:

(a) obtaining a modified blastocyst;

(b) implanting the modified blastocyst into a pseudopregnant animal; and

(c) allowing the blastocyst to develop into a ~~non-human~~ chimeric animal,

wherein the modified blastocyst comprises a blastocyst from a first animal that comprises modified stem cell from a second animal,

wherein the modified stem cell comprises a stem cell that comprises a plurality of chromosomes and at least a first heterologous nucleic acid molecule,

wherein the modified stem cell can differentiate into a plurality of cell types; the first heterologous nucleic acid molecule is integrated into a chromosome of the modified stem

cell at a first locus whereby, upon differentiation of the modified stem cell, the first heterologous nucleic acid is expressed in the plurality of differentiated cell types,

wherein the first heterologous nucleic acid molecule encodes a first polypeptide selected from secreted proteins, extracellular domains of transmembrane proteins, and active fragments thereof; and

wherein the first polypeptide is other than beta-galactosidase and a recombinase.

89. (currently amended): A method of making a ~~non-human~~ chimeric animal comprising the steps of:

(a) obtaining a modified blastocyst;

(b) implanting the modified blastocyst into a pseudopregnant ~~non-human~~ animal; and

(c) allowing the blastocyst to develop into a ~~non-human~~ chimeric animal,

wherein the modified blastocyst comprises a blastocyst from a first animal that comprises one or more modified stem cells from a second animal,

wherein the modified stem cell comprises a stem cell that comprises a plurality of chromosomes and at least a first heterologous nucleic acid molecule,

wherein the modified stem cell can differentiate into a plurality of cell types; the first heterologous nucleic acid molecule is integrated into a chromosome of the modified stem cell at a first locus whereby, upon differentiation of the modified stem cell, the first heterologous nucleic acid is expressed in the plurality of differentiated cell types,

wherein the first heterologous nucleic acid molecule encodes a first polypeptide selected from single transmembrane proteins, multi-transmembrane proteins, kinases, proteases, phosphatases, phosphodiesterases, kinesins, histone deacetylases, hormone receptors, ubiquitin E3 ligases, and active fragments thereof, and

wherein the first polypeptide is other than beta-galactosidase and a recombinase.

90. (currently amended): A method of making a ~~non-human~~ chimeric animal comprising the steps of:

(a) obtaining a modified blastocyst;

(b) implanting the modified blastocyst into a pseudopregnant ~~non-human~~ animal; and

(c) allowing the blastocyst to develop into a ~~non-human~~ chimeric animal,

wherein the modified blastocyst comprises a blastocyst from a first animal that comprises one or more modified stem cells from a second animal,

wherein the modified stem cell comprises a plurality of chromosomes and at least a first heterologous nucleic acid molecule,

wherein the modified stem cell can differentiate into a plurality of cell types; the first heterologous nucleic acid molecule is integrated into a chromosome of the modified stem cell at a first locus whereby, upon differentiation of the modified stem cell, the first heterologous nucleic acid is expressed in the plurality of differentiated cell types,

wherein the first heterologous nucleic acid molecule encodes a first polypeptide that is an episomal plasmid maintenance molecule or an active fragment thereof, and

wherein the first polypeptide is other than beta-galactosidase and a recombinase.

91. (canceled)
92. (canceled)
93. (canceled)
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101. (canceled)
102. (canceled)
103. (canceled)
104. (canceled)
105. (canceled)

106. (canceled)

107. (currently amended): A method of determining an *in vivo* effect of a first polypeptide in an animal, comprising the steps of:

(a) obtaining a ~~non-human~~ chimeric animal of ~~any one of claims 54-64~~ claim 54; and

(b) observing the ~~non-human~~ chimeric animal for phenotypic, histologic, or physiologic changes.

108. (original): A method of determining an *in vitro* effect of a first polypeptide on a cell, comprising the steps of:

(a) obtaining a modified stem cell of claim 1; and

(b) observing the modified stem cell for phenotypic, histologic, or physiologic changes.

109. (canceled)

110. (original): A method for production of a heterologous polypeptide comprising the steps of:

(a) obtaining a modified stem cell of claim 1; and

(b) allowing the modified stem cell to proliferate whereby, the heterologous polypeptide is produced.

111. (original): The method of claim 110, wherein the heterologous nucleic acid molecule of the modified stem cell is under regulatory control of a first promoter, wherein the first promoter is inducible, comprising the step of activating the inducible promoter.

112. (original): The method of claim 110, wherein the heterologous polypeptide is a transmembrane protein, and the modified stem cell expresses the transmembrane protein on its cell surface.

113. (original): The method of claim 110, wherein the heterologous polypeptide is a secreted protein, and the modified stem cell secretes the secreted protein into a growth medium.

114. (canceled)

115. (original): A library comprising a plurality of modified stem cells of claim 1, wherein the plurality of modified stem cells comprise modified stem cells, wherein the heterologous nucleic acid molecule encodes a first member of a family of proteins or an active fragment thereof, and second modified stem cells, wherein the heterologous nucleic acid molecule encodes a second member of the family of proteins or an active fragment thereof.

116. (canceled)

117. (canceled)

118. (canceled)

119. (canceled)

120. (canceled)

121. (original): A composition comprising a first modified and at least a second modified stem cell,

wherein the first modified stem cell comprises at least a first heterologous nucleic acid molecule that encodes a first polypeptide, and the second modified stem cell comprises at least a second heterologous nucleic acid molecule that encodes a second polypeptide,

wherein the first polypeptide encodes a secreted factor and the second polypeptide encodes a receptor,

wherein the first nucleic acid integrates at a first locus of a chromosome of the first modified stem cell and the second nucleic acid integrates at a second locus of a chromosome of the second modified stem cell, and

wherein the first and second locus are identical.

122. (original): The composition of claim 121, wherein the first locus is selected from ROSA26, ROSA5, ROSA11, and G3BP(BT5).

123. (canceled)

124. (canceled)

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168. (canceled)

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170. (canceled)

171. (canceled)

172. (canceled)

173. (canceled)

174. (canceled)

175. (canceled)

176. (currently amended): A chimeric ~~non-human~~ animal stem cell comprising an ~~non-human~~ animal stem cell and at least one first heterologous nucleic acid sequence, wherein the first heterologous nucleic acid sequence encodes a first human polypeptide other than β-galactosidase, wherein the first heterologous nucleic acid sequence is inserted at a first locus of a chromosome of the ~~non-human~~ animal, and wherein insertion of the first heterologous nucleic acid sequence at the first locus enables expression of the polypeptide in the chimeric stem cell in both a differentiated and undifferentiated state.

177. (canceled)

178. (canceled)

179. (currently amended): The chimeric ~~non-human~~ animal stem cell of claim 176, wherein the first ~~human~~ polypeptide is a ~~human~~ secreted polypeptide.

180. (canceled)

181. (canceled)

182. (canceled)

183. (canceled)

184. (canceled)

185. (canceled)

186. (currently amended): The chimeric ~~non-human~~ animal stem cell of claim 179, wherein the stem cell is differentiated.

187. (canceled)

188. (canceled)

189. (canceled)

190. (canceled)

191. (canceled)

192. (canceled)

193. (canceled)

194. (canceled)

195. (canceled)

196. (canceled)

197. (canceled)

198. (canceled)

199. (canceled)

200. (canceled)

201. (canceled)

202. (currently amended): A chimeric ~~non-human~~ embryo, fetus, or animal produced from the chimeric non-human animal stem cell of claim ~~124, or the chimeric non-human blastocyst of claim 198~~ 176, or a progeny thereof.

203. (canceled)

204. (canceled)

205. (canceled)

206. (canceled)

207. (original): One or more cells derived from the animal of claim 202.

208. (original): A non-human animal comprising at least one first heterologous polynucleotide that encodes a first heterologous polypeptide, wherein the animal is homozygous with respect to the first heterologous nucleic acid sequence, and the animal is produced from the chimeric non-human animal of claim 202 or a progeny thereof.

209. (canceled)

210. (canceled)

211. (canceled)

212. (original): One or more cells derived from the animal of claim 208.

213. (original): A chimeric non-human animal resulting from a cross between at least one first animal that is a chimeric non-human animal of claim 202 or a progeny thereof, or a first non-human animal comprising a first heterologous nucleic acid sequence that encodes a first heterologous polypeptide, wherein the animal is homozygous with respect to the first heterologous polynucleotide, and the animal is produced from a chimeric non human animal or a progeny thereof; and a second animal that is a non-human animal or a progeny of said second animal.

214. (original): One or more cells derived from the animal of claim 213.

215. (original): The non-human animal of claim 213, wherein the second animal provides an animal model of disease.

216. (canceled)

217. (canceled)

218. (canceled)

219. (canceled)

220. (canceled)

221. (canceled)

222. (canceled)

223. (canceled)

224. (canceled)

225. (original): Isolated tissues derived from the non-human animal of claim 202.

226. (canceled)

227. (canceled)

228. (canceled)

229. (canceled)

230. (canceled)

231. (canceled)

232. (canceled)

233. (canceled)

234. (canceled)

235. (canceled)

236. (canceled)

237. (canceled)

238. (original): A method of determining gene function *in vivo* comprising the steps of

(a) providing a modified embryonic stem cell, wherein the modified embryonic stem cell comprises an introduced gene, wherein the introduced gene is a silencer and is present at a particular locus of the modified embryonic stem cell;

(b) introducing the modified embryonic stem cell into a blastocyst to form a modified blastocyst;

(c) implanting the modified blastocyst into an animal to produce a chimeric embryo, fetus or animal that expresses the introduced gene in more than one tissue; and

(d) determining or observing the effect of the introduced gene on the embryo, fetus, or animal.

239. (original): The method of claim 238, wherein the silencer is an RNAi, antisense, or ribozyme.

240. (canceled)

241. (canceled)

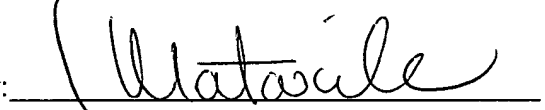
242. (original): One or more cell lines derived from a chimeric embryo, fetus, or animal of claim 238 or a progeny of such.

Applicants respectfully submit that entry of this amendment is therefore proper. Entry of this Preliminary Amendment and the timely examination and allowance of the pending claims is courteously requested. If there is any fee due in connection with the filing of this Preliminary Amendment, please charge the fee to our Deposit Account No. 06-0916.

Respectfully submitted,

FINNEGAN, HENDERSON,
FARABOW,
GARRETT & DUNNER,
L.L.P.

Dated: December 6, 2004

By: 

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